

Haemoglobin and packed cell volume (PCV) of high-fat diet/streptozotocine-induced diabetic Wistar Rats treated with ethanol extract of a herbal mixture (Aju Mbaise).

ABSTRACT

Aim: This study was carried out to evaluate the effect of ethanol extract of Aju Mbaise herbal mixture on some haematological indices of diabetic Wistar albino rats.

Sample: Packed cell volume (PCV) and haemoglobin (Hb) concentration was estimated in diabetic rats treated with ethanol extract of Aju Mbaise herbal mixture.

Study design: In the course of the experiment, fifty-four (54) rats with initial weight range of 30 – 40g were grouped into 6 of 9 rats per group. The first group served as the normal control (NC) while the remaining five groups were induced with diabetes type 2 using high-fat diet for 8 weeks and streptozotocin at 35mg/kg body weight. Group II served as the diabetic control while the remaining groups (III, IV, V & VI) were treated with metformin and three different concentrations of the plant extract respectively.

Place and Duration of Study: The study was carried out in the Animal house of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, between July 2018 and January 2019.

Methodology: The haemoglobin and packed cell volume were estimated after 4th, 8th and 12th week of treatment using MINDRAY Auto-Haematology analyzer.

Results: From the results obtained, it was observed that the diabetic control group has a PCV and haemoglobin concentration that is significantly ($P<.05$) lower when compared to that of the normal control group and the other treated groups.

Conclusion: The study has shown that Aju Mbaise herbal mixture is a haematopoietic agent as it had the tendency to synthesize blood cells.

Keywords:

Aju Mbaise, Haematopoietic, Streptozotocin, Metformin and Mindray.

Introduction

Diabetes mellitus (DM) is a chronic widely spread human autoimmune disease associated with abnormally high level of sugar (glucose) in the blood. According to [1], diabetes mellitus describes

34 a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with
35 disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion,
36 insulin action, or both. Diabetes mellitus is of two major types; the type-1 DM also known as the
37 insulin dependent diabetes mellitus (IDDM) and the type-2 DM also known as the non-insulin
38 dependent diabetes mellitus (NIDDM). The third possible type of DM is the gestational diabetes
39 mellitus, which arises during pregnancy and may disappear with or without treatment after
40 pregnancy. According to Nishimura [2], a critical evaluation of mortality patterns by race showed
41 that blacks had a higher mortality rate than whites, and it also affect individuals of all ages [1].
42 According to [3], patients with diabetes mellitus show a significant derangement in various
43 haematological parameters. A high prevalence of anaemia was identified in type-2 diabetic patients
44 [4]. Thus, about 27% of diabetics' patients are anaemic [5]. Diabetes mellitus has been regulated
45 with the use of some antidiabetic drugs, including insulin and other biochemical hypoglycemic
46 agents such as tolbutamide, phenformin, troglitazone, rosiglitazone and repaglinide. According to
47 [6], these therapies has shown to control the blood glucose level only when they are regularly
48 administered, but also are tedious and have several undesirable side effects and fail to significantly
49 alter the course of diabetic complications [7]. Thus, gave rise to the use of medicinal plant for the
50 treatment and/or control of diabetes mellitus. The resource plant 'Aju Mbaise' is a traditional
51 medicine, composed of combination of different leaves, roots and trunk of medicinal tree wrapped
52 together and is taken in the form of concoctions. [8], reported that this plant decoction has good
53 amount of quality proteins, minerals and vitamins, and also possesses antibacterial activity. The
54 ability of this plant to demonstrate such quality is dependent on the accumulated natural products,
55 biologically active materials and ingredients found in them. Due to the phytochemicals, minerals,
56 vitamins, and nutrients present in the individual plants that make up Aju Mbaise, there is a
57 likelihood that this plant possesses haematopoietic activity. This research work encompasses the
58 induction and treatment of type-2 diabetes mellitus which is a chronic condition that affects the
59 glucose metabolism in the body. To achieve the outlined objectives of this research work, high fat
60 diet (HFD) and streptozotocin was used to induce type 2 diabetes mellitus, while the ethanol extract
61 of Aju Mbaise plant samples was used for the treatment of the diabetes for a period of twelve (12)
62 weeks, and the plant's effect on packed cell volume (PCV), and haemoglobin (Hb) concentration of
63 female Wistar albino rats was measured with an auto-haematology analyser.

64

65 **Materials and Methods**

66 **Procurement of Experimental Animals**

67 A total of fifty-four (54) female Wistar albino rats was used for this experiment. They were
68 acquired from the Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu
69 State. They were housed in the animal house of the Department of Pharmacology, University of
70 Port Harcourt, Rivers State, Nigeria. The animals were left for 1 week to acclimatize to the
71 laboratory conditions during which they were administered normal feed (Top feeds- grower's
72 mash) and clean water. The animals were later grouped into six groups of nine animals per group.

73 **Collection of Plant Samples**

74 Fresh samples of the plants that make up Aju Mbaise were collected at Obodo Ujichi, Ahiazu and
75 Amuzi, Ahiara Towns, both in Aboh Mbaise L.G.A, of Imo State, Nigeria. The plants include
76 *Cnestis ferruginea*, *Xylopi aethiopica*, *Uvaria chamae*, *Palisota hirsuta*, *Scleria sp.*, *Napoleona*
77 *imperialis*, *Dialium guineense*, *Combretum racemosum*, and *Heterotis rotundifolia* respectively. The
78 fresh plants after collection were air-dried, cut into small pieces and blended before the extraction
79 process. The extraction was done with ethanol as the solvent.

80 **Preparation of Extract**

81 The whole plants parts (leaves and stem) were washed, air dried and blended to a powdered form.
82 Powdered sample weighing 1,000g was soaked in 3,000ml of 95% ethanol for 48 hours after which
83 it was sieved using a muslin cloth and afterwards filtered through a Whatmann filter paper No. 1.
84 The filtrate was concentrated using a rotary evaporator at 45° C and afterwards placed on a
85 thermostatic water bath for further drying. The concentrate (paste) was collected, weighed, kept in
86 sterile bottles and stored at 4° C until usage.

87 **Preparation of high fat diet**

88 The high fat diet was compounded according to the method of [9], using standard laboratory chow
89 (Top feed) growers mash, lard and sucrose in the ratio of 3:1:1 respectively. The diet was
90 carefully homogenized, then fed to the animals (groups II to VI) with the exception of the normal
91 control group.

92 **Induction of Type 2 Diabetes mellitus**

93 A single dose intraperitoneal injection of 35mgkg⁻¹ body weight of streptozotocin was used to
94 induce type 2 diabetes mellitus to the experimental animals in groups II to VI. Respective doses of
95 the streptozotocin were dissolved in 0.2ml normal saline per rat before administration. The
96 development of hyperglycaemia in the rats was allowed for 7 days after the streptozotocin injection
97 and fasting blood glucose levels checked before the commencement of treatment. The treatment
98 was done by oral administration of the drugs and extracts using intra-gastric gavage daily for 12
99 weeks. At every 4 weeks interval, 3 animals were fasted overnight, anaesthetized, sacrificed and
100 blood samples collected using EDTA for haematological analyses.

101 **Method of analysis**

102 The PCV and haemoglobin concentration was estimated using a MINDRAY auto-haematology
103 analyzer. The blood sample was mixed with a sample mixer for 2-5 minutes, after which it was
104 introduced into the auto-analyzer and 250µl of the blood sample was aspirated by the analyzer. The
105 haematological parameters to be analyzed were selected and the machine was allowed to run for 2-3
106 minutes after which the results were printed out from a printer.

107 **Experimental Design**

108 The experimental female Wistar albino rats were grouped into six groups of nine animals. Group I
109 animals (normal control) are non-diabetic, Group II (diabetic control) animals are diabetic rats that
110 remained untreated throughout the experimental period. Groups III to VI animals were made
111 diabetic but were treated with a known antidiabetic drug (7.2mgkg⁻¹ body weight metformin), and
112 three different concentrations (500mg/kg, 250mg/kg & 100mg/kg) of the herbal mixture extract.

113 **Ethical Approval**

114 All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23,
115 revised 1985) were followed, as well as specific national laws where applicable. All experiments
116 have been examined and approved by the appropriate ethics committee.

117 **Statistical Analysis**

118 Data were presented as Mean ± standard error of mean. The results were analyzed using one way
119 ANOVA and Tukey HSD Post Hoc Test with significance at $P < 0.05$.

120 **Results**

121 The result of the PCV and haemoglobin level were presented below (Tables 1 & 2).

122 **Table 1: Effect of Aju Mbaise herbal mixture extract on Packed Cells Volume (PCV) in**
123 **HFD/STZ-induced female diabetic rats**

| Groups | PCV (%) | | |
|--------|-------------------------|-------------------------|------------|
| | Week 4 | Week 8 | Week 12 |
| NC | 42.00±2.08 ^b | 43.00±2.08 ^b | 39.33±0.88 |
| DC | 35.33±2.03 ^a | 36.00±1.53 ^a | 36.33±1.45 |

| | | | |
|---------------|--------------------------|--------------------------|------------|
| Metformin | 40.67±0.88 ^b | 41.67±0.33 ^b | 38.33±1.45 |
| 500mg Extract | 39.33±0.88 ^{ab} | 38.33±2.19 ^{ab} | 38.67±0.67 |
| 250mg Extract | 38.33±2.03 ^{ab} | 40.33±1.20 ^{ab} | 37.33±1.86 |
| 100mg Extract | 39.67±0.67 ^{ab} | 38.33±0.67 ^{ab} | 38.00±1.00 |

124 Values represent Mean ± SEM, and n = 3. Groups with different Superscript(s) are significantly
 125 different at p<0.05, while groups with same superscript(s) are not.
 126

127 **Key:** NC= Normal control; DC= Diabetic control; **Metformin**= Treated with metformin; **500mg**
 128 **Extract** = Treated with 500mg/kg of the extract of the cocktail herbal mixture; **250mg Extract** =
 129 Treated with 250mg/kg of the extract of the cocktail herbal mixture; **100mg Extract** = Treated with
 130 100mg/kg of the extract of the cocktail herbal mixture.

131 **Table 2: Effect of Aju Mbaise herbal mixture extract on haemoglobin (Hb) level in HFD/STZ-**
 132 **induced female diabetic rats**

| Groups | Hb (g/dl) | | |
|---------------|------------|--------------------------|------------|
| | Week 4 | Week 8 | Week 12 |
| NC | 12.67±0.88 | 13.33±0.33 ^b | 12.97±0.09 |
| DC | 10.67±1.20 | 9.67±1.76 ^a | 11.30±0.98 |
| Metformin | 11.43±0.98 | 11.00±0.58 ^{ab} | 11.77±0.96 |
| 500mg Extract | 11.30±0.74 | 11.63±0.74 ^{ab} | 11.87±0.83 |
| 250mg Extract | 11.47±0.45 | 10.57±0.66 ^{ab} | 11.17±0.69 |
| 100mg Extract | 10.93±1.03 | 9.60±0.31 ^a | 11.53±1.47 |

133 Values represent Mean ± SEM, and n = 3. Groups with different Superscript(s) are significantly
 134 different at p<0.05, while groups with same superscript(s) are not.
 135

136 **Key:** NC= Normal control; DC= Diabetic control; **Metformin**= Treated with metformin; **500mg**
 137 **Extract** = Treated with 500mg/kg of the extract of the cocktail herbal mixture; **250mg Extract** =
 138 Treated with 250mg/kg of the extract of the cocktail herbal mixture; **100mg Extract** = Treated with
 139 100mg/kg of the extract of the cocktail herbal mixture.

140 **Discussion**

141 The present study showed that diabetes mellitus has effect on some haematological parameters such
 142 as packed cell volume (PCV) and haemoglobin. According to [10], a wide range of haematology

143 laboratory values change significantly in patients with diabetes. In this study, PCV which is an
144 index of anaemia was significantly lower among diabetic subjects when compared to the non-
145 diabetic controls and the treated animals. Anaemia is known to be prevalent among diabetic's
146 patients and may also be significant in determining the outcome of heart failure and hypoxia-
147 induced organ damage in patients with diabetes [11]. Due to the incomplete life span of
148 erythrocytes in diabetic condition, Hb concentration which depends on plasma volume and
149 erythrocyte mass is lowered [12]. Also according to [13], anaemia has been seen to occur in
150 diabetic mellitus condition and this may be due to the increase in non-enzymatic glycosylation of
151 erythrocyte membrane proteins which correlates with hyperglycemia. Oxidation of these
152 glycosylated membrane proteins can cause an increase in the production of lipid peroxides causing
153 haemolysis of the red blood cells [14]. [15], reported that diabetes related anaemia has been
154 observed in diabetic nephropathy, and about 20% of those with type 2 diabetes may eventually
155 develop kidney damage and later kidney failure. It was also suggested that diabetic anaemia may be
156 because of insufficient androgen releasing function of adrenal glands or less erythropoietin
157 concentration [16]. In other study of [17], it was also suggested that anaemia has a high prevalence
158 in type 2 diabetic patients and it has high correlation with kidney disorders. [18], also reported that
159 diabetes incorporation with kidney nephropathy can cause anaemia. Anaemia may also be
160 significant in determining the outcome of heart failure and hypoxia-induced organ damage in
161 diabetes. While several factors contribute to the increased prevalence of anaemia in diabetes, the
162 failure of the kidney to increase erythropoietin in response to falling haemoglobin appears to be the
163 dominant factor. In this study, Wistar albino rats were induced diabetes mellitus type-2 with
164 administration of high fat diet and a low dose (35 mg/kg) injection of streptozotocin. The
165 experimental animals were treated with a standard antidiabetic drug (metformin) and three (3)
166 different concentrations of extract of cocktail of Aju Mbaise herbal mixture. From the result of the
167 experiment presented in Table 1, it was observed that there was a significant decrease in PCV of the
168 diabetic control animals when compared to that of the normal control and diabetic animals treated
169 with metformin and the three different concentrations of the herbal mixture extract, throughout the
170 experimental period. This was also noted in the Haemoglobin result presented in Table 2; where
171 there was a significant decrease in haemoglobin concentration of the diabetic control animals when
172 compared to that of the normal control and diabetic animals treated with metformin and the three
173 different concentrations of the herbal mixture extract. The results obtained from this study is in
174 agreement with other studies by [19, 20], that found decreased hematological parameters (PCV, Hb,
175 and RBC) in type 2 diabetic patients. Similarly, a previous study observed a lower mean values of
176 RBC, Hb, PCV and MCHC in diabetic patients when compared to the control group, indicating the

177 presence of anaemia in the former group [21]. There may also be decreased PCV in patients with
178 type-1 diabetes because of the higher levels of blood glucose which can potentially cause
179 intracellular dehydration. This finding does indicates that PVC may not be a good marker for the
180 monitoring of anaemia among type-1 diabetic patients. Thus, it may be better to use haemoglobin
181 level in the monitoring of anaemia among patients with type-1 diabetes. The ability of the Aju
182 Mbaise herbal mixture extract to improve/increase the PCV and haemoglobin levels in diabetic
183 animals could be attributed to the phytochemicals as well as the minerals and vitamins content of
184 the herbal mixture.

185 **Conclusion**

186 This study has shown that the mean values of PCV and haemoglobin are lower among diabetic
187 Wistar albino rats when compared to the non-diabetic controls and the diabetic rats treated with
188 metformin and ethanolic extract of Aju Mbaise herbal mixture respectively. It was observed that the
189 administration of the cocktail herbal mixture of Aju Mbaise increased PCV and haemoglobin level
190 in diabetic rats. Thus, this herbal mixture is a potential erythropoietic agent.

191 **Competing Interests**

192 Authors have declared that no competing interests exist.

193 **References**

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